

Claims

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1. *Helicobacter pylori* protein in a substantially purified form, capable of being obtained from an *H. pylori* membrane fraction, and whose molecular weight after electrophoresis on a 10% polyacrylamide gel in the presence of SDS appears of the order of 54, 50, 32-35 or 30 kDa; provided that when the molecular weight is 54 kDa, the protein does not react with an anti-catalase antiserum.
2. Protein according to Claim 1, whose apparent molecular weight is of the order of 54 kDa and which is capable of being obtained by a process in which:
- (i) the *H. pylori* bacteria are extracted with 1% n-octyl β -D glucopyranoside, followed by centrifugation;
 - (ii) a bacterial pellet is recovered and it is treated with lysozyme and subjected to sonication, followed by centrifugation;
 - (iii) a centrifugation pellet is recovered and it is subjected to washing with 20 mM Tris-HCl buffer pH 7.5, followed by centrifugation;
 - (iv) the membrane fraction consisting of the centrifugation pellet is recovered and it is resuspended in aqueous medium;
 - (v) the membrane fraction is subjected to an anion-exchange chromatography on a Q-Sepharose column in a 0 - 0.5 M NaCl gradient, followed by washing in 1 M NaCl;
 - (vi) the fraction eluted at the start of washing in 1 M NaCl is recovered and it is subjected to an anion-exchange chromatography on a DEAE-Sepharose column, in a 0 - 0.5 M NaCl gradient; and
 - (vii) the fraction eluted in 0.1 - 0.25 M NaCl is recovered.
3. Protein according to Claim 1, whose apparent molecular weight is of the order of 50 kDa and which is capable of being obtained by a process in which:
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pylori bacteria are extracted with β -D glucopyranoside, followed by centrifugation; the supernatant is recovered and the residual bacterial pellet is recovered with lysozyme and sonication, followed by centrifugation. The supernatant from the first centrifugation pellet is recovered and subjected to washing with 20 mM Tris, pH 7.5, followed by centrifugation. The supernatant from the second centrifugation membrane fraction consisting of the supernatant from the first centrifugation pellet is recovered and subjected to dialysis in aqueous medium; the dialysate is subjected to ion exchange chromatography on a Q-Sepharose column with a 0 - 0.5 M NaCl gradient, and the protein is eluted in 1 M NaCl; the protein is then subjected to ion exchange chromatography on a Q-Sepharose column, and the protein is eluted at the start of the gradient. The protein is recovered and it is subjected to ion exchange chromatography on a Q-Sepharose column, in a 0 - 0.5 M NaCl gradient, and the protein is eluted in 0.3 - 0.5 M NaCl.


According to Claim 3, where the amino acid sequence of the protein is determined according to Claim 1, the molecular weight of the protein is of the order of 30 kDa, and the protein is obtained by a process in which the bacteria are extracted with β -D glucopyranoside, followed by centrifugation; the supernatant is recovered and the residual bacterial pellet is recovered with lysozyme and sonication, followed by centrifugation. The supernatant from the first centrifugation pellet is recovered and subjected to washing with 20 mM Tris, pH 7.5, followed by centrifugation.

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pylori bacteria are extracted with β -D glucopyranoside, followed by centrifugation; the supernatant liquid is recovered and washed with 20 mM Tris-HCl buffer, pH 7.5, followed by centrifugation. The membrane fraction consisting of the supernatant liquid after centrifugation pellet is recovered and resuspended in aqueous medium; the membrane fraction is subjected to ion exchange chromatography on a Q-Sepharose column, in a 0 - 0.5 M NaCl gradient, where the protein fraction eluted at the start of the gradient is recovered and it is subjected to ion exchange chromatography on a Q-Sepharose column, in a 0 - 0.5 M NaCl gradient, where the protein fraction eluted in 0.3 - 0.5 M NaCl is recovered.

According to Claim 3, where the amino acid sequence of the protein is determined according to Claim 1, where the molecular weight is of the order of 30 kDa, the protein obtained by a process in accordance with Claim 1, where pylori bacteria are extracted with β -D glucopyranoside, followed by centrifugation; the supernatant liquid is recovered and washed with 20 mM Tris-HCl buffer, pH 7.5, followed by centrifugation.

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- pylori bacteria are extracted with β -D glucopyranoside, followed by centrifugation; the supernatant liquid is recovered and washed with 20 mM Tris-HCl buffer, pH 7.5, followed by centrifugation. The membrane fraction consisting of the supernatant liquid after centrifugation pellet is recovered and added in aqueous medium; the membrane fraction is subjected to ion exchange chromatography on a Q-Sepharose column, in a 0 - 0.5 M NaCl gradient, where the protein fraction eluted at the start of the gradient is recovered and it is subjected to ion exchange chromatography on a Q-Sepharose column, in a 0 - 0.5 M NaCl gradient, where the protein fraction eluted in 0.3 - 0.5 M NaCl gradient is recovered.
- According to Claim 3, wherein the amino acid sequence of the protein is as follows:
- According to Claim 1, wherein the molecular weight of the protein is of the order of 30 kDa and the protein is obtained by a process involving the extraction of Helicobacter pylori bacteria with β -D glucopyranoside, followed by centrifugation; the supernatant liquid is recovered and washed with 20 mM Tris-HCl buffer, pH 7.5, followed by centrifugation.

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- (iv) the membrane fraction consisting of the centrifugation pellet is recovered and it is resuspended in aqueous medium;
 - (v) the membrane fraction is subjected to an anion-exchange chromatography on a Q-Sepharose column in a 0 - 0.5 M NaCl gradient;
 - (vi) the fraction eluted in 0.28 - 0.35 M NaCl is recovered and it is subjected to an anion-exchange chromatography on a DEAE-Sepharose column, in a 0 - 0.5 M NaCl gradient; and
 - (vii) the fraction corresponding to the direct eluate is recovered (absence of NaCl).

6. Protein according to Claim 1, whose apparent molecular weight is of the order of 32-35 kDa and which is capable of being obtained by a process in which:

- (i) the *H. pylori* bacteria are extracted with 1% n-octyl β -D glucopyranoside, followed by centrifugation;
- (ii) a bacterial pellet is recovered and it is treated with lysozyme and subjected to sonication, followed by centrifugation;
- (iii) a centrifugation pellet is recovered and it is subjected to washing with 20 mM Tris-HCl buffer pH 7.5, followed by centrifugation;
- (iv) the membrane fraction consisting of the centrifugation pellet is recovered and it is resuspended in aqueous medium, advantageously in carbonate buffer pH 9.5;
- (v) the suspension obtained in (iv) is centrifuged at about 200,000 x g and the supernatant is recovered;
- (vi) the pH of the supernatant obtained in (v) is reduced to about pH 7, advantageously by dialysing against phosphate buffer pH 7;
- (vii) the preparation obtained in (vi) is subjected to a cation-exchange chromatography on an SP-Sepharose column in a 0 - 0.5 M NaCl gradient, advantageously in a phosphate buffer pH 7; and

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(vii) the fraction eluted in 0.26 - 0.31 M NaCl is recovered.

7. *Helicobacter* protein or a polypeptide derived from the protein by fragmentation and/or mutation, in a substantially purified form, which is capable of being recognized by an antiserum raised against a protein according to claim 1.

8. Pharmaceutical composition for the prevention or treatment of an *H. pylori* infection, which comprises as active ingredient a protein or a polypeptide according to claim 1.

9. Pharmaceutical composition for the prevention or treatment of an *H. pylori* infection, which comprises as active ingredient a protein or a polypeptide according to claim 7.

10. Monospecific antibody capable of recognizing a protein or a polypeptide according to claim 1.

11. Monospecific antibody capable of recognizing a protein or a polypeptide according to claim 7.

12. Pharmaceutical composition intended for the prevention or treatment of an *H. pylori* infection, which comprises as active ingredient a monospecific antibody according to claim 10.

13. Pharmaceutical composition intended for the prevention or treatment of an *H. pylori* infection, which comprises as active ingredient a monospecific antibody according to claim 11.

14. Diagnostic method which makes it possible to detect the presence of *Helicobacter* in a biological sample, according to which the biological sample is brought into contact with an antibody according to claim 10 so that an immune complex forms, the unbound material is optionally removed and the immune complex formed between the sample and the antibody is detected.

15. Diagnostic method which makes it possible to detect the presence of antibodies to *Helicobacter* in a biological sample, according to which the biological sample is brought into contact with a polypeptide of claim 7 so that an immune complex forms, the unbound material is optionally removed and the immune complex formed between the sample and the polypeptide is detected.

16. Process for the purification of a protein or of a polypeptide according to claim 1 from a biological sample, according to which the biological sample is subjected to an affinity chromatography using a monospecific antibody according to claim 10.

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